graft
Review

A Look at the History of Bone Marrow Stromal Cells

The Legacy of Alexander Friedenstein

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Abstract

Alexander Friedenstein first described bone marrow stromal cells (BMSCs) more than 30 years ago. From that time on, he and his coworkers extensively studied BMSCs in a number of species, including humans, and characterized them in great detail. Techniques were developed for the determination of BMSC colony forming efficiency, strain generation, and in vivo transplantation. BMSC differentiation into bone, cartilage, myelosupporting stroma, and adipose tissue was carefully characterized, a theory of BMSCs as stromal stem cells was introduced, and the concept of determined and inducible osteogenic precursor cells was elaborated. Significant progress was made towards understanding problems of BMSC migration and of fibroblast precursors in blood. In this short review, we discuss the insights into BMSC biology that represent only a part of Dr. Friedenstein's ample legacy.

Introduction

The significance of bone marrow stromal cells (BMSCs) is now being increasingly appreciated, and their potential applications for tissue regeneration and gene therapy are being extensively explored. However, much of today's excitement about the capabilities of these cells is based on the lifetime's work of a single scientist, who over 30 years ago brought them into the limelight. Alexander Jakovlevich Friedenstein (1924-1997) was born in Kiev, Ukraine, and lived in Moscow after 1928 (Fig. 1 and Fig. 2). He received his M.D. and Ph.D. from the Moscow Medical Institute, and his D.Sc. from the NF Gamaleya Institute for Microbiology and Epidemiology where he organized and, for three decades, headed the Laboratory of Immunomorphology. Despite the long years of now infamous stagnation, short supply and severely restricted personal contacts, the Laboratory exemplified scientific creativity and productivity. During his years as Professor of Histology at the Moscow University and the Head of Laboratory, Dr. Friedenstein continued to be a true bench-worker, personally doing tissue culture and transplantation experiments, preparing tissue sections, performing immunochemical stainings, etc. The depth and fundamental nature of his knowledge, combined with his extremely creative and original mind impressed all who knew Dr. Friedenstein. His apt criticism was never destructive, and his advice was always encouraging and constructive. His acute sense of humor together with a profound knowledge in a diversity of fields, from world history to music to psychology, made him a one-in-a-million personality. Dr. Friedenstein published over 100 scientific papers and five monographs. His ideas, findings, and experimental approaches have fundamentally influenced many areas of mesenchymal cell biology. Below, we briefly review some of these areas.

BMSCs In Vitro

In contrast to many other tissues, bone marrow and lymphoid tissues can be dissociated into individual cells by soft mechanical treatment. When plated in vitro at low cell densities, mechanically prepared single cell suspensions of marrow give rise to discrete adherent colonies of fibroblast-like cells. By 10-12 days, most colonies consist of thousands of cells that were referred to as marrow stromal fibroblasts, or bone marrow stromal cells (BMSCs).[1,2] BMSCs share many, but not all, properties of fibroblastic cells of other tissues; some characteristics distinguish them from endothelial cells and macrophages. Each BMSC colony is formed by proliferation of a single precursor cell referred to as a colony-forming unit—fibroblast (CFU-F). In steady state conditions in vivo, most CFU-Fs are in Go stage of the cell cycle and enter into S phase between 24 and 60 hours after plating.[2,3]

The identity of CFU-Fs in situ is not yet entirely clear. Morphological data point to cells with elusive morphology and multiple names: adventitial cells, reticular cells, stromal fibroblasts, undifferentiated mesenchymal cells, Westen-Bainton cells. In the post-natal marrow, these cells form both an adventitial (outer) coating of the sinusoid wall and branching extravascular meshwork that provides the physical

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Fig. 1. Alexander Friedenstein with his wife, Elena Luria, in the mid-60s.

substrate for medullary myelopoiesis. Westen-Bainton cells are characterized by extensive, elongated cell processes and membrane-bound alkaline phosphatase activity. They express low levels of collagen types I and III and osteonectin, and are, therefore, considered 'fibroblastic';[4,5] it should be noted, however, that marrow stroma is relatively devoid of extracellular matrix. In different animal species, varying proportions of these cells survive mechanical dissociation and plating in vitro. Of those that survive and attach to the culture dishes, the great majority begin to proliferate to form BMSC colonies; these cells are, in fact, CFU-Fs.[6]

Dr. Friedenstein and his associates characterized BMSC colony formation in a number of species, including mouse, rat, guinea pig, rabbit, hamster and humans, in terms of cell kinetics, radiation sensitivity, growth factor requirements and CFU-F concentration.[7-10] The latter was expressed as colony forming efficiency (CFE), or number of BMSC colonies per $1x10^5$ marrow nucleated cells in the original marrow cell suspension. In animals under physiological conditions, CFE remains relatively stable; it is, however, somewhat age-dependent, and can be significantly altered by experimental procedures, such as acute bleeding, irradiation or curettage. In humans, CFE is also relatively constant; in normal bone marrow not diluted with peripheral blood, it is between 20 and 70 per $1x10^5$ marrow cells.[11] If CFE values from normal human donors are much lower than those mentioned above, it may indicate a significant loss of CFU-Fs due either to suboptimal media and sera, or to inadequate cell separation and culture techniques. Under Dr. Friedenstein's direction, CFE was studied in a broad range of hematological disorders and was found to be abnormal in some, such as acute lymphoblastic leukemia, lymphocytoma and refractory anemia. Continuing this approach, we recently demonstrated dramatic CFE changes in certain skeletal pathologies and metabolic disorders, such as severe achondroplasia, fibrous dysplasia of bone and McCune-Albright syndrome, and total lipodystrophy. These data further support Dr. Friedenstein's view that CFE is an important parameter reflecting some aspect of skeletal status, and point to a role of CFU-Fs in the pathophysiology of disorders of bone and bone marrow. They also demonstrate that CFE may be a useful diagnostic tool, in particular, for classification of vaguely delineated disorders, including osteoporosis.

After BMSC colonies are formed, the cells can be removed from the culture dish with trypsin and expanded by multiple passages. In Dr. Friedenstein's laboratory, techniques were developed for generating BMSC strains from guinea pigs, rabbits, rats and humans. These cells can undergo over 25 passages in vitro (more than 50 cell doublings), demonstrating a high capacity for self replication; billions of BMSCs can be thus generated from a limited amount of starting material, such as 1 ml of bone

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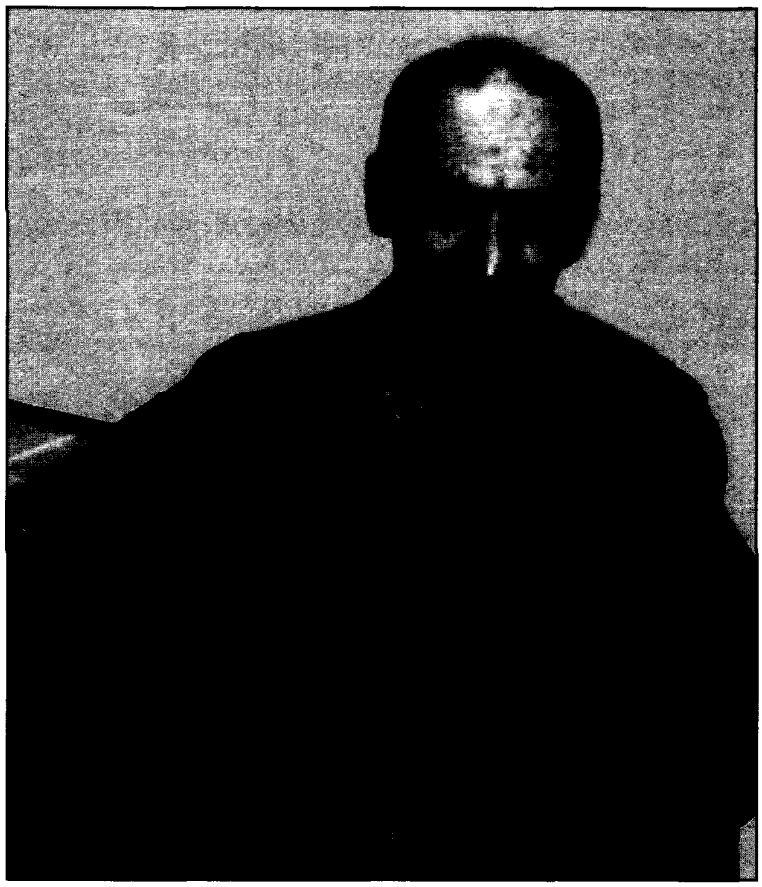

Fig. 2. Dr. Alexander Friedenstein, 1993

In transplants of both BMSCs and marrow tissue, CFU-Fs, bone cells, and hematopoiesis-supporting stroma are of donor origin, while hematopoietic cells are of recipient origin.

marrow aspirate. After several passages of guinea pig, rabbit and human cultures, virtually pure BMSC populations are present,[7,8,12] while in mouse cultures, special efforts are required to remove abundant macrophages. During continuous ex vivo expansion, BMSCs preserve a diploid karyotype and, under standard culture conditions, show no signs of differentiation.

BMSC In Vivo Transplantation

In vivo transplantation has been both a major tool for studying BMSC differentiation potential and the most important area of BMSC application. In this part, we will review discrete BMSC transplantation (heterotopic and orthotopic), as opposed to systemic injection.

The connection between the bone marrow and osteogenesis was first observed in the 19th century, and revived in the 1960s when Dr. Friedenstein described bone formation by transplanted marrow fragments.[13,14] Later, he demonstrated that it is BMSCs that constitute osteogenic elements within the bone marrow tissue. Experimental BMSC transplantation has been conducted heterotopically by using either closed or open systems. In the closed system, cells are transplanted, mostly intraperitoneally, within diffusion chambers made of Millipore filters (0.22 μm or 0.45 μm porosity), such that the transplants receive nutrients but have no direct contact with the host cells. No special analysis is therefore required to determine the origin of tissues formed within the chambers. BMSCs transplanted in closed systems form bone, cartilage, fibrous tissue, and sometimes fat. Bone is developed next to the filters, while cartilage is found towards the center of the chamber suggesting that BMSC differentiation may be influenced by the nutritional environment, including oxygen gradients (reviewed in ref. 4). This conclusion is supported by the observation that in chambers with a narrow gap between the filters (0.1 mm),

only bone is formed, while in chambers with a wider gap (2 mm), both bone and cartilage are developed.[12]

In the open system, transplanted cells have direct contact with recipient tissues. Most often, transplantation into subcutaneous sites or under the kidney capsule is performed. To avoid immunological rejection, syngeneic or immunocompromised recipients must be used. When BMSCs are transplanted in such a way, rapid vascularization of the graft is followed by osteogenesis,[8] resulting in extensive bone formation in transplants of mouse, rat, rabbit, guinea pig, dog and human BMSCs (reviewed in ref. 15). In addition to bone, fibrous tissue, hematopoiesis-supporting reticular stroma and associated adipocytes are also formed; cartilage is only rarely found in open BMSC transplants. Vast fields of hematopoiesis are developed in close proximity to the new bone; in the absence of new bone, hematopoiesis is never observed. Judging by both the differential cell counts and the numbers of hematopoietic precursors, the new hematopoietic tissue formed in the transplants is equivalent to the normal orthotopic bone marrow. In transplants of both BMSCs and marrow tissue, CFU-Fs, bone cells, and hematopoiesis-supporting stroma are of donor origin, while hematopoietic cells are of recipient origin.[14,16-18]

The extent of bone formed by transplanted BMSCs varies broadly depending on the transplantation conditions. No bone is developed when BMSCs are injected, subcutaneously or intramuscularly, as a cell suspension. Likewise, no bone is formed when BMSCs are transplanted as a cell pellet without a vehicle, or within rapidly resorbed vehicles. Apparently, in order to form bone, transplanted BMSCs require the presence of an organized framework to which they can attach and which can sustain their growth for periods long enough for osteogenic events to occur. Rodent BMSCs form good bone when transplanted within collagen sponges.[19,20] Human BMSCs, however, are more sensitive to the nature of transplantation vehicle: they form very poor bone or no bone at all when transplanted in vehicles such as collagen sponges, human demineralized bone matrix, polyvinyl sponges and poly(L-lactic acid). Human BMSCs consistently form bone in vehicles containing hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic in the form of blocks, powder or Collagraft™ (mixture of ceramic powder with bovine collagen type I). In these transplants, extensive bone is developed on the surface of HA/TCP; it is mostly lamellar in structure and well mineralized, and surrounds abundant hematopoietic cells. In HA/TCP powder, with or without fibrin as a secondary matrix, more extensive bone is formed than in HA/TCP blocks.[17] We are now working towards further optimization of human BMSC transplantation system by varying HA/TCP particle size, minimizing cell contact with xenogeneic sera during ex vivo expansion, etc.

The new bone and hematopoietic tissue formed in BMSC heterotopic transplants show no signs of degeneration or senescence for at least two years post-transplantation and are, essentially, self-maintained. Extra hematopoietic territories of desirable size and location could be thus created that might benefit certain hematological conditions. In conjunction with genetic engineering, permanent yet easily extractable heterotopic ossicles could be devised to continuously release proteins of interest into circulation. Another important application of this approach is based on the fact that in heterotopic transplants of BMSCs, both normal and pathological, composition of the original tissue is recreated. Rabbit BMSCs derived from red and yellow marrow form heterotopic ossicles containing stroma with abundant hematopoiesis and hypocellular stroma with mainly fat cells, respectively, thus mirroring the composition of their original site (reviewed in ref.15). BMSCs from transgenic mice deficient for membrane-bound matrix metalloproteinase reproduce upon transplantation two related defects: impairment of osteogenic capacity and impairment of collagenolytic activity.[21] In transplants of BMSCs from patients with fibrous dysplasia of bone and the McCune-Albright syndrome, abnormal features of fibrous dysplastic lesion of bone are recapitulated.[22] These findings establish a means to create mouse models of human bone and marrow disorders, with the potential for development of new methods for treatment.

Use of BMSCs in direct orthotopic transplantation is a more straightforward application. Currently, autografts of cortical or cancellous bone are most commonly employed for treatment of bone defects. This method is, however, limited by autograft availability and donor site morbidity. Direct orthotopic total bone marrow transplantation is another option that is being actively explored. Transplantation of BMSCs has further advantages over transplantation of the whole marrow cell population. First, large numbers of BMSCs can be generated from a single marrow aspirate, thus avoiding the necessity for harvesting large amounts of autologous marrow. Secondly, bone formation by transplanted BMSCs generally occurs more

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rapidly and is more extensive than by total marrow cells. Finally, ex vivo expanded BMSCs can be genetically or otherwise manipulated and selected. An important step towards their clinical application is accomplished by the finding that syngeneic BMSCs can successfully regenerate critical-size bone defects involving femoral bones in rat, and calvarial bones in mouse. Furthermore, in immunocompromised animals, the two types of bone defects can be healed by human BMSCs (reviewed in ref. 15). The first clinical attempt performed under Dr. Friedenstein's supervision demonstrated an accelerated healing of long-lasting nonunions and long bone defects into which ex vivo expanded human autologous BMSCs had been transplanted.[23] These combined data offer a reasonable hope that BMSC transplantation will play a significant role in the repair of skeletal defects caused by tumor removal, bone infection, congenital malformation and fracture nonunion.

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High self-replicating capacity and multipotentiality of BMSCs allowed Dr. Friedenstein and Dr. Owen to formulate a concept according to which the CFU-F populations comprise multipotential mesenchymal precursors.[4,6] Acknowledging some stem cell properties of the CFU-F, this theory emphasizes fundamental differences between the stromal system, with its slow renewal rate, and the hematopoietic system that has extensive cell renewal capacity.[2] In particular, the true "stem-cell" character of the CFU-F, i.e., its ability for continuous self-renewal, has never been definitively demonstrated. Further, in contrast to the hematopoietic stem cells that are equally multipotential, CFU-Fs are not homogenous as relates to their differentiation capacities. Indeed, when individual colonies of mouse, guinea pig and rabbit BMSCs were transplanted in vivo, only some of them formed bone and hematopoiesis-supporting stroma, including adipocytes (in open systems), or bone and cartilage (in closed systems). The rest of the colonies formed bone or fibrous tissue.[6,7,12,24] When human individual-colony derived BMSC strains were transplanted, 23.5% of them formed extensive bone accompanied by hematopoiesis, 35.3% formed less abundant bone without hematopoiesis, and the remainder formed just fibrous tissue.[18] This data demonstrates that CFU-F populations are heterogeneous: some CFU-Fs are multipotential precursor cells, while others have more limited potential, being committed to particular differentiation pathways.[4]

BMSCs and Cell Migration

Dr. Friedenstein was a strong proponent of the view that CFU-Fs do not naturally migrate from one part of bone marrow to another and, accordingly, their engraftment does not usually occur following systemic infusion. His own data demonstrate that in mouse bone marrow heterotopic transplants, the initial donor BMSCs are retained, and no additional recipient BMSCs are recruited.[7,14,16,24] In mouse radiation chimeras reconstituted by injection of non-irradiated marrow cells, BMSCs regenerate without participation of donor cells, despite the selective advantage the latter have over their irradiated counterparts.[16] Likewise, many independent groups have demonstrated that in recipients of sex-mismatched, HLA-identical marrow allotransplants, all BMSCs uniformly originate from the host marrow; this result is not influenced by the conditioning regimen, the numbers of injected marrow cells, and the nature of pre-existing disease (reviewed in ref. 15). Conversely, a number of studies demonstrated evidence for the persistence of systemically injected BMSCs. This subject therefore remains highly controversial; its ultimate importance guarantees that many future attempts will be undertaken to solve it. Indeed, in a variety of generalized bone disorders, such as osteoporosis and Osteogenesis Imperfecta, it is not feasible to transplant genetically engineered BMSCs directly into all affected bones, so their distribution via the circulatory system would be highly desirable. If Dr. Friedenstein's views hold up, extra steps may be necessary in order to achieve the engraftment of injected BMSCs.

The subject of stromal cell migration may be approached from another direction, which is the question of fibroblast precursors in blood. Transformation of blood leukocytes into fibroblasts is one of the oldest and most controversial concepts in cell biology. One of the first observations of this phenomenon was made by Dr. Alexander Maximow in 1902. In his last work published in 1928, the development of fibroblasts in cultures of guinea pig blood cells was characterized in detail (reviewed in ref. 24). Thirty years later, Dr. John Paul demonstrated that human blood cells also could give rise to fibroblast-like cells. The major obstacle that complicates the study of blood borne fibroblasts (BBFs) is the question of whether these cells are in fact present among the blood cells, or result from connective tissue contamination occurring during the process of blood collection. Evidence for both views exists. A significant step towards resolving the controversy was achieved by the elegant

experiments conducted by Dr. Friedenstein together with his wife, Dr. Elena Luria. Equal volumes of guinea pig blood were obtained either by a single cardiac puncture or by multiple punctures. If fibroblastic precursors had originated from the heart wall, BBF colony numbers would have been higher in the latter case than in the former. In fact, however, colony numbers were equal in both settings,[25] demonstrating that BBFs are genuinely present among blood cells.

Further noteworthy insights into the problem of stromal cell migration came from Dr. Friedenstein's studies of induced osteogenesis. Analyzing bone induction by transitional epithelium of urinary bladder, he developed a concept of determined and inducible osteogenic precursor cells. The former, determined osteogenic precursor cell (DOPC), include BMSCs and other spontaneously osteogenic cell populations of skeletal origin. The latter, inducible osteogenic precursor cell (IOPC), comprise a number of extraskeletal populations, including thymic mesenchymal stromal cells, splenic stromal cells and fibroblastic cells grown from peritoneal exudates. These cells require inductive stimuli to start osteogenic differentiation.[2,3,8] Of note, while our current understanding of osteogenic molecules has advanced significantly, not much progress in characterization of cells inducible to osteogenesis has been achieved since Dr. Friedenstein's studies. In one of his experiments, Dr. Friedenstein implanted epithelium of urinary bladder into a guinea pig's tibia, locally irradiated with 1000 to 5000 rad. Despite pronounced radiation injury, the course of bone induction was unaffected demonstrating that IOPC were not local but migrated from non-irradiated areas. When guinea pig blood cells were transplanted together with the inducer in a diffusion chamber, bone was formed inside, directly proving that IOPC circulate in blood.[3] Recently, we have found that a proportion of mouse, guinea pig, rabbit and human BBF colonies form bone spontaneously after in vivo transplantation; thus, DOPC are present among the blood cells, as well. These findings represent a step towards understanding BBF biology, but fall short of solving the mystery of their origin, destination, and function. It remains to be determined what relationship, if any, exists between the circulating osteogenic precursors and BMSCs, and whether the former, in fact, participate in osteogenic events, such as physiological bone turnover, callus formation and ectopic calcification. Without this knowledge, the existence of BBFs by itself does not prove that BMSCs do migrate.[24]

Conclusion

Since the late 60s, Dr. Friedenstein and his coworkers extensively studied BMSCs of a number of species, including humans, and comprehensively characterized them. Techniques were developed, that essentially have not been modified since, for BMSC colony forming assay, strain generation and in vivo transplantation. BMSC differentiation towards bone, cartilage, myelosupportive stroma and adipose tissue was thoroughly described, and a theory of BMSCs as multipotential precursor cells was developed. It should be recognized that while the newcomers to the field may use different names and some different methodologies, they are still dealing with BMSCs.

With his fondness for new ideas and novel approaches, Alexander Friedenstein would have loved to see the cells he once discovered being used for tissue engineering, analyzed by microarray, used for the creation of cDNA libraries, etc. In his own research, Dr. Friedenstein was confined to more traditional, classical techniques. Yet he accomplished a great deal, and his legacy, as that of other scientists of his stature, will potentially cast unique insights into our understanding of mesenchymal cell biology.

Abbreviations

BBF, blood borne fibroblast; BMSC, bone marrow stromal cell; CFE, colony forming efficiency; CFU-F, colony forming unit—fibroblast; DOPC, determined osteogenic precursor cell; HA/TCP, hydroxyapatite/tricalcium phosphate; IOPC, inducible osteogenic precursor cell.